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Effects of pre-sowing treatments on seed germination and seedling growth of *Glycine max(L.) Merrill*

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***Abstract*— The study assessed the effects of some pre-sowing seed treatments on seed germination and seedling growth rate of three varieties of Glycine max– TGX 1935-3F (variety 1), TGX 1448-2E (variety 2) and TGX 1951-3F (variety 3). The treatments included four different concentrations of sulphuric acid (0.1, 0.2, 0.3, 0.6mole/dm^3) termed T1, T2, T3 and T4 respectively, for five minutes and three hot water temperature variations (40, 60, and 80^{0}C) termed T5, T6 and T7 respectively, for twenty minutes. Germination was monitored as emergence of radicle and plant growth parameters were also measured. Treatments T4 (93.33%, 86.67%, 96.67%) and T7 (90.00%, 83.33%, 93.33%) had the highest germination percentages in the three varieties. Plant height in T2, T3 and T4was significantly different ($p < 0.05$) from the control in all acid pre-treatments for all varieties; for hot water pre-treatment, plant height in varieties 1 and 3 were significantly ($p < 0.05$)different from the control in all treatments. Furthermore, T6 and T7 (variety 2) were also significantly different from the control. Number of leaves showed significant difference from the control in T3, T4 (variety 1), T4 (variety 2), and T2, T3, and T4 respectively. Additionally, T5, T6 and T7 were significantly different from the control in varieties 1 and 2. Leaf area was significantly different ($p < 0.05$)from the control in T1, T2, T3, T4 (varieties 1 and 3), and in T4 (variety 2). Moreover, T5, T6 and T7 were significantly different from the control in varieties 1 and 3, and in T7 in variety 2. Number of buds was significantly different ($p < 0.05$) in T2, T4 (variety 1), and in T3 and T4 in variety 3. There was also significant difference in the number of buds in T5, T6 and T7 in variety 3. The results suggested treatments T4 and T7 had the best overall effect on germination and overall growth, and hence hereby recommended.*

***Keywords*— Glycine max, Dormancy, Acid treatment, Hot water treatment, Cellular Phase Transition.**

I. INTRODUCTION

Glycine max (L.) Merrill commonly known as soybean, is a species of legume native to East Asia and grown for its numerous uses (Felker and Bandurski, 1979; Burkhill, 1994). Almost all types of soil support its growth except deep sand with poor water retention (Iwe, 2003). Soybean is used commercially as human food, and livestock feed and for the extraction of oil. It has the adaptability to fix nitrogen. It is composed of about 40% protein and 20% oil. This composition ranked it highest in protein content and second highest in terms of oil content food crops(Iwe, 2003). Because of its importance in providing many uses and services, it has attracted some attention.

However, one of the problems with this species is that some varieties produce some hard seed coats that do notreadily imbibe water even for prolong period of soaking (Shao*etal*., 2007). The cuticle of such seeds contain disproportionately high amount of hydroxylated fatty acid, which makes germination difficult. The seeds therefore require treatment before sowing to enhance germination and improved seedling growth (Shao *et al*., 2007).Germination is a multifaceted event, involving up regulation of genes promoting germination and down regulation of genes promoting dormancy (Matilla and Matilla-Vazquez, 2008). Germination has been shown to be affected by the endogenous seed concentration and soil levels of plant growth hormones (Graeber *et al*., 2012; Kucera *et al*., 2005). Furthermore, activation of proteinase triggers the mobilization of storage proteins (Tiedemann *et al*., 2001). Other enzymes such as carboxyl peptidase and amino peptidase have also been implicated in the emergence of radicle and plumule (Finch-Savage and Leubner-Metzger, 2006). In addition, environmental factors such as acidity, temperature, salinity have been elucidated to affect hormonal balance. Gibberellic acid (GA) enhance seed germination by promoting enzymes that inhibit Abscisic acid (ABA) pathway and subsequently, ABA concentration (Atia*et al*., 2009). Ethylene and GA antagonize ABA during dormancy initiation, termination and germination, but the

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crosstalk has not been fully elucidated (Rodríguez-Gacio*et al*., 2009; Miransari and Smith, 2014; Matilla and Matilla-Vazquez, 2008). Although dormancy is controlled at the molecular level (Graeber *et al*., 2012), the physical characteristics of the testa, pericarp and endosperm have profound effects on germination (Kucera *et al*., 2005).

Various studies have employed different methods of breaking or reducing dormancy and improving germination rate including cold water (Adeola and Dada, 1983; Eze and Orele, 1987), hot water (Awodola, 1994; Adewusi and Ladipo, 2000; Otegbeye and Momodu, 2002) and acid treatment (Ibrahim and Kalu, 2006). According to Umar(2005), these treatments made the seed coat permeable and enhanced germination. The purpose of this study was to investigate the effects of pre-sowing seed treatment on germination and seedling growth of *Glycine max*.

II. MATERIALS AND METHODS

The study was conducted at the nursery section of the Department of Biological Sciences, University of Agriculture Makurdi, Nigeria.

Plant Materials

Seeds of three varieties of *G. max*were obtained from the Seed Technology Centre, University of Agriculture Makurdi, Nigeria. The three varieties of *G. max* used were– TGX 1935-3F, TGX 1448-2E and TGX 1951-3F; termed variety 1, 2 and 3 respectively. The varieties were hand-picked to ensure uniformly large-size, and seeds were checked to ensure they were physically undamaged.Physical identification of the varieties was done – with TGX 1935-3F, TGX 1448-2E, and TGX 1951-3F having brown, yellow and black seed coats respectively.

Experimental Design and Treatments

A 3x3x8 Factorial experiment (3 varieties, 3 replicates and 8 treatments) laid out in a Completely Randomized Design was adopted for the study. There were seventreatments (and a control), with three replicates for each treatment per variety, and ten seeds were assessed for each replicate.The treatments included acid scarification by immersing the seeds in sulphuric acid (H_2SO_4) at different concentrations (0.1, 0.2, 0.3 and 0.6 mol/dm^3), termed Treatments T1, T2, T3 and T4 respectively, for 5 minutes. Also, seeds were immersed in hot water of varying temperatures (40,60, and 80^{o}C) for 20 minutes, termed Treatments T5, T6 and T7 respectively. Acid treated seeds were washed with distilled water to ensure complete removal of trace acid before planting in poly pots. Control seeds were sown without any pretreatment.All seeds were planted at 2cm depth in poly pots. The study lastedfor five weeks. Data on germination were collected in week one, and data on growth parameters for four weeks. Growth parameters such as plant height, number of leaves, leaf area, and number of buds were evaluated. Germination percentage was calculated using the equation described by ISTA (1999).

Statistical Analyses

Statistical analyses were done using Minitab 16 Statistical Software. One-way analysis of variance (ANOVA) was used to compare multiple means at 95% confidence interval. Mean separation was done using Least Significant Difference (LSD) and$p<0.05$ was considered statistically significant.

III. RESULTS

Germination Percentage:

Table 1 shows the germination percentage of the three varieties of *G. max* seeds subjected to pre-sowing acid and hot water treatments. The results indicated increases in germination percentage with increases in acid concentrations and water temperatures respectively in the three varieties, while T4 and T7 yielded the highest germination percentage.

Table.1: Germination percentage of three varieties of G. max. seedstreated with sulphuric acid and hot water pre-sowing treatments

Treatments	Germination %		
	Variety 1	Variety 2	Variety 3
Control	60.00	53.33	66.67
T1	63.33	70.00	80.00
T2	66.67	80.00	83.33
T3	70.00	83.33	86.67
T4	93.33	86.67	96.67
T5	73.33	66.67	60.00
T6	76.67	76.67	80.00
T7	90.00	83.33	93.33

Variety 1 (TGX 1935-3F);Variety 2 (TGX 1951-3F);Variety 3 (TGX 1448-2E); T1 (0.1 mol/dm^3); T2 (0.2 mol/dm^3); T3 (0.3 mol/dm^3); and T4 (0.6 mol/dm^3). T5 (40 ^{o}C); T6 (60 ^{o}C); and T7 (80 ^{o}C).

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Growth Parameters of Acid Treatments:

Variety 1 - TGX 1935-3F

Significant differences ($p<0.05$) from the control were observed in plant height in treatments T1, T2, T3 and T4 (Figure 1). The tallest plantswere seen in treatment T4. The number of leaves in treatments T3 and T4 were significantly different (Figure 1) from the control. Also, the leaf area in treatments T1, T2, T3 and T4 was significantly different from the control (Figure 1). Furthermore, significant differences from the control were observed in the number of buds of treatments T2 and T4.

Variety 2 - TGX 1951-3F:

Significant differences ($p<0.05$) from the control were observed in plant height of treatments T2, T3 and T4 (Figure 1). The number of leaves and leaf area of treatment T4 were also significantly different from the control (Figure 1).

Variety 3 - TGX 1448-2E:

Significant differences ($p<0.05$) from the control were observed in the plant height and number of leaves (Figure 1) of treatments T2, T3 and T4. The leaf area of all treatments (T1 to T4) was significantly different (table 4) from the control. Additionally, the number of buds (Figure 1) of treatments T3 and T4 were significantly different from the control.

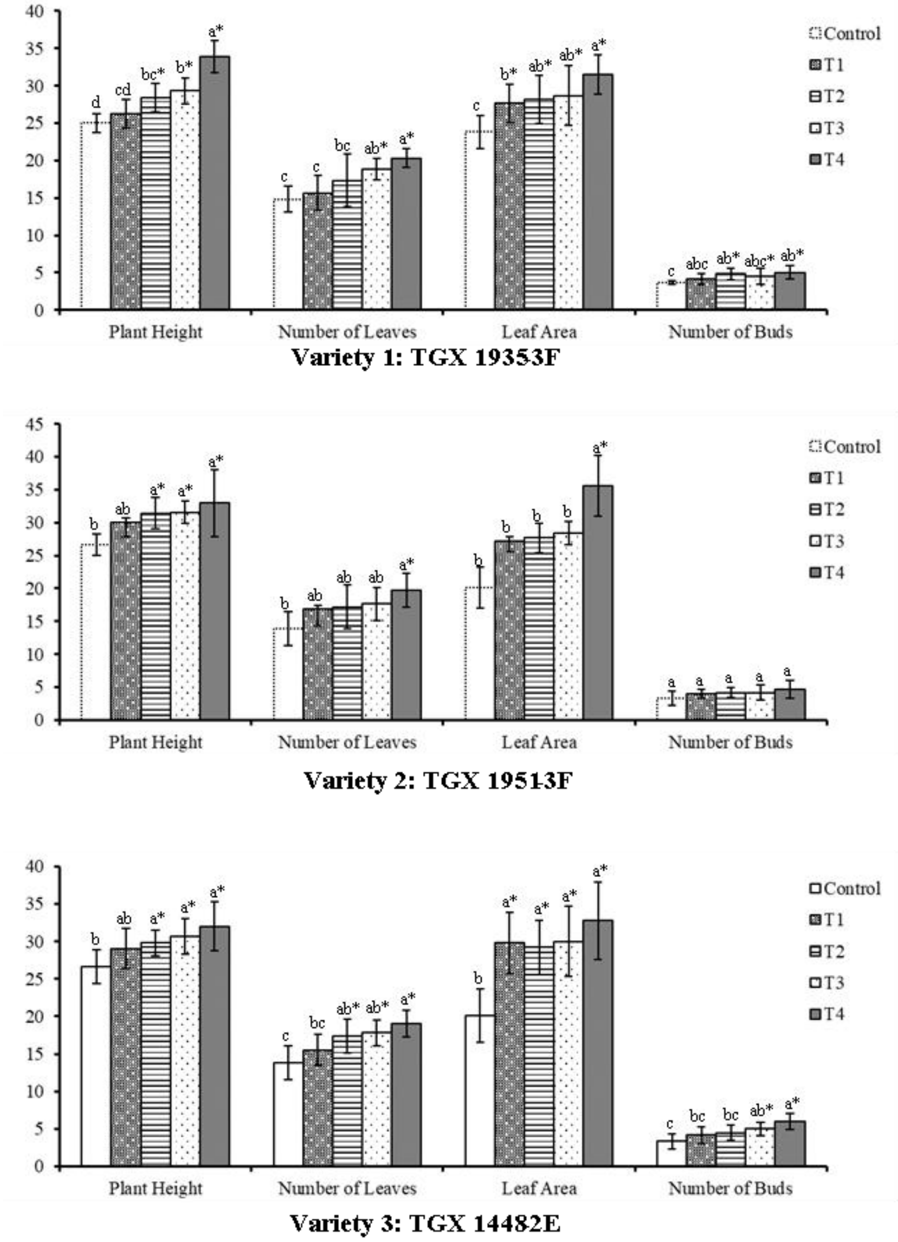

*Fig.1: Effects of sulphuric acid pre-sowing seed treatments on growth parameters of three varieties of G. max.Vertical bars represent means; error bars represent ± standard deviation; n = 10; * = significantly different from the control ($p<0.05$). Means with the same superscript are not significantly different. Plant Height (cm); Leaf Area (cm^2);T1 (0.1 mol/dm^3); T2 (0.2 mol/dm^3); T3 (0.3 mol/dm^3); and T4 (0.6 mol/dm^3) acid pre sowing treatments for 5 minutes.*

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Growth Parameters of Hot Water Treatments:

Variety 1 - TGX 1935-3F

Plant height, number of leaves and leaf area in all hot water treatments, T5, T6 and T7, were significantly different($p<0.05$) from the control (Figure 2).

Variety 2 - TGX 1951-3F:

Plant heights of treatments T6 and T7 were significantly different from the control (Figure 2). Furthermore, there was significant difference from the control in plant height and leaf area of Treatment T7. Number of leaves and buds were not significantly different from the control in all treatments.

Variety 3 - TGX 1448-2E

All treatments showed significant differences from the control ($p<0.05$) in the plant height, number of leaves, leaf area and number of buds (Figure 2).

IV. DISCUSSION

Increased germination percentage in pre-sowing treated seeds in this study is in line with similar findings reported by (Amira and Mohammed, 2013) whose work showed increased germination percentage of *Cassia fistula* seeds treated with sulphuric acid and hot water and (Aliero, 2004) on the positive effect of sulphuric acid on germination of *Parkiabiglobosa*. Additionally, Agbogidi*et al*. (2007) demonstrated that acid treatment of *Dacryodes edulis* significantly improved germination, plant height, number of leaves and leaf size with increasing time of acid pre-sowing exposure. Similaraly, Moosavi*et al*., (2014) observed improved germination percentage, vegetative and reproductive traits of 8-hour seed water pre-treatment of soybeans. These studies have highlighted the role of softening the seed coat in seed germination.

The pre-sowing treatments appear to soften the seed coat, thereby triggering a cascade of molecular events that led to the breaking of dormancy; hence, higher germination percentages and head start for seedlings whose seeds were subjected to such pre-sowing treatments. Furthermore, the germination and growth parameters measured in the higher acid and hot water pre-sowing treatments (T4 and T7) showed better overall performance suggesting the efficiency of the treatments in breaking dormancy and improving seedling growth.

Advances in molecular biology – metabolomics, transcriptomic, genomics, proteomics, bioinformatics – allow biologists to create genome-wide network models revealing in-depth insights into seed germination and regulation of plant cellular phase transitions (Bassel*et al*., 2011; Linkies*et al*., 2010). Such studies have shed light on genes involved in germination and hence, deciphered the genetic process of germination. Bassel*et al* (2011) elucidated the possible co-evolution of germination and dormancy, and the potential interplay with cellular-phase transition genes, suggesting that dormancy arose as an evolutionary adaptive stage by linking genetic pathways involved in cellular phase transition and abiotic stress.

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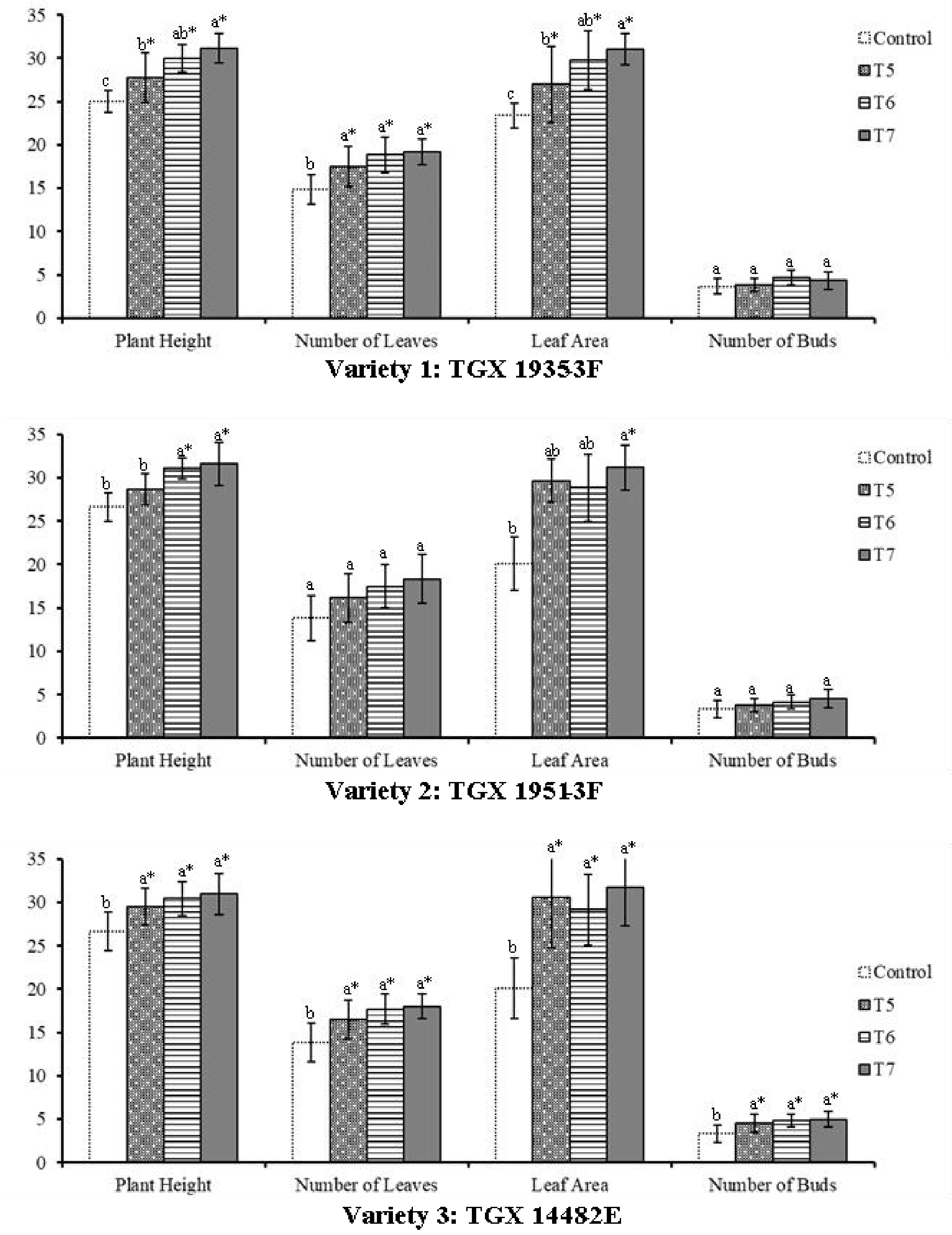

*Fig.2: Effects of hot water pre-sowing seed treatments on growth parameters of three varieties of G. max. Vertical bars represent means; error bars represent ± standard error. n = 10; * significantly different from the control ($p<0.05$). Means with the same superscript are not significantly different. Plant Height (cm); Leaf Area (cm^2); T5 (40 °C); T6 (60 °C); and T7 (80 °C) hot water pre-sowing treatments for 20 minutes.*

V. CONCLUSION

The study suggests pre-sowing treatments have profound effect on germination and seedling development of soybean by softening the seed coat and triggering molecular cascades involved in germination, breaking dormancy and cellular phase transition, leading to higher germination percentages and better seedling growth.

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